

Genetic correction and analysis of induced pluripotent stem cells from a patient with gyrate atrophy.

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Public Summary:

Gene-corrected patient-specific induced pluripotent stem (iPS) cells offer a unique approach to gene therapy. Here, we begin to assess whether the mutational load acquired during gene correction of iPS cells is compatible with use in the treatment of genetic causes of retinal degenerative disease. We isolated iPS cells free of transgene sequences from a patient with gyrate atrophy caused by a point mutation in the gene encoding ornithine-δ-aminotransferase (OAT) and used homologous recombination to correct the genetic defect. Cytogenetic analysis, array comparative genomic hybridization (aCGH), and exome sequencing were performed to assess the genomic integrity of an iPS cell line after three sequential clonal events: initial reprogramming, gene targeting, and subsequent removal of a selection cassette. No abnormalities were detected after standard G-band metaphase analysis. However, aCGH and exome sequencing identified two deletions, one amplification, and nine mutations in protein coding regions in the initial iPS cell clone. Except for the targeted correction of the single nucleotide in the OAT locus and a single synonymous base-pair change, no additional mutations or copy number variation were identified in iPS cells after the two subsequent clonal events. These findings confirm that iPS cells themselves may carry a significant mutational load at initial isolation, but that the clonal events and prolonged cultured required for correction of a genetic defect can be accomplished without a substantial increase in mutational burden.

Scientific Abstract:

Gene-corrected patient-specific induced pluripotent stem (iPS) cells offer a unique approach to gene therapy. Here, we begin to assess whether the mutational load acquired during gene correction of iPS cells is compatible with use in the treatment of genetic causes of retinal degenerative disease. We isolated iPS cells free of transgene sequences from a patient with gyrate atrophy caused by a point mutation in the gene encoding ornithine-delta-aminotransferase (OAT) and used homologous recombination to correct the genetic defect. Cytogenetic analysis, array comparative genomic hybridization (aCGH), and exome sequencing were performed to assess the genomic integrity of an iPS cell line after three sequential clonal events: initial reprogramming, gene targeting, and subsequent removal of a selection cassette. No abnormalities were detected after standard G-band metaphase analysis. However, aCGH and exome sequencing identified two deletions, one amplification, and nine mutations in protein coding regions in the initial iPS cell clone. Except for the targeted correction of the single nucleotide in the OAT locus and a single synonymous base-pair change, no additional mutations or copy number variation were identified in iPS cells after the two subsequent clonal events. These findings confirm that iPS cells themselves may carry a significant mutational load at initial isolation, but that the clonal events and prolonged cultured required for correction of a genetic defect can be accomplished without a substantial increase in mutational burden.

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